16. REPEATED DOSE TOXICITY

A. Subchronic Toxicity:

i) 90 Day Toxicity study in Rats (1984):

Test substance:	Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydro cinnamate) Purity > 99%; Batch = EN 42222.12 CAS No. 41484-35-9
Method:	This carried out based on the OECD Guidelines for testing of chemicals, sub-chronic oral toxicity-Rodent: 90-day study No. 408, adopted May 12, 1981 and in accordance with the OECD Principles of Good Laboratory Practice (GLP), adopted May 12, 1981 by the OECD council. The experiment was carried out under specific pathogen free (SPF) standard laboratory conditions. Rats were housed in groups of five in Macrolon cages type 4 with standardized granulated soft bedding. Diet and water were allowed ad libitum. The animal room was air conditioned and maintained at a temperature of 22 ± 2^{0}C, relative humidity of 55 ± 10%, with 16-20 air changes/ hour and illuminated for 12 hours/ day. The compound was administered at a dose level of 0, 60, 200, 600, and 2000 ppm by mixing in the food. Clinical symptoms and mortalities were recorded daily; food consumption and body weights were recorded weekly. An histopathological examination was carried out on the following organs: skin, mammary area, spleen, mesenteric lymph node, axillary lymph node, sternum with bone marrow, femur with joint, skeletal muscle, trachea, lung, heart, aorta, submandibular salivary gland, liver, pancreas, oesophagus, stomach, small intestine, large intestine, kidney, urinary bladder, prostate, seminal vesicle, testis, epididymis, uterus, ovary, pituitary gland, adrenal gland, thyroid with parathyroid gland, thymus, peripheral nerve, brain, spinal cord, eye with optic nerve, orbital gland, extraorbital lacrimal gland, organs and tissues showing macroscopic changes.[1]
Species/strain:	Rat, Tif: RAIf (SPF) / F3-hybrid of RII 1/Tif x RII 2/Tif
Initial age:	4 weeks
Initial Body weight:	96 – 101 g, males 95 – 102 g, females
No. of animals:	20 males and 20 females/ group (total 200)
Route of administration:	Oral in the diet

Exposure period:	93 - 103 days
Dose:	0, 60, 200, 600, and 2000 mg/kg [ppm] in food
GLP:	yes
Year:	1984
Results:	The calculated mean daily intake of the test substance was approximately 4.4, 12.5, 39, and 138 mg/kg bw in males and 4.5, 13, 40, and 140 mg/kg bw in females. Body weight gains, food consumption, specific food consumption in relation to body weight, water consumption were similar to that of the control group. No mortalities occurred. No clinical symptoms and no signs of local and / or systemic toxicity were observed. Ophthalmic inspections and hearing examinations performed before and towards the end of the application period revealed no evidence of a reaction to the treatment. Hematology: The findings in the haematological investigation were unremarkable. Occasional intergroup differences were considered incidental in nature and not related to the treatment with the test substance. Blood chemistry values are comparable to control group. Organ Weights and Ratios: Mean organ weight and ratios are presented in the following summary tables. Both absolute and relative liver weight showed a dose dependent increase reaching the level of statistical significance in treated male groups 3, 4, and 5 (200, 600, and 2000 ppm) and in treated female group 5(2000 ppm). Additional statistically significant differences in organ weights, as indicated by asterisks in the following mean tables, between treated and control groups were noted. Since no systematic pattern emerged, except a trend to higher kidney and testis weights in males and thyroid weight in males and females at higher dosages – which corresponds to the higher weight of exsanguinated body in these groups – these differences were attributed to spontaneous variation rather than to the treatment.

Mean Organ Weight and Ratios (As a Percentage of Body - and Brain Weight)

Table 1

Data for Male Rats

ORGANS	DOSE IN PPM										
	0.0		60.0		200.0		600.0		2000.0		TREND
	NO.	MEAN	NO.	MEAN	NO.	MEAN	NO.	MEAN	NO.	MEAN	
+Body	20	469.079	20	466.364	19	475.399	20	480.414	20	485.079	
+Brain	20	2.444	20	2.462	20	2.448	20	2.415	20	2.449	
Brain / Body	20	0.529	20	0.533	19	0.518	20	0.507	20	0.510	
+Heart	19	1.439	20	1.415	20	1.380	20	1.426	20	1.410*	
Heart / Body	19	0.303	20	0.304	19	0.294	20	0.298	20	0.293	
Heart / Brain	19	59.099	20	57.545	20	56.547	20	59.188	20	57.700	
+Liver	20	14.445	20	16.014	20	17.135*	20	18.575*	20	19.802*	---->
Liver / Body	20	3.064	20	3.439*	19	3.652*	20	3.872*	20	4.097*	---->
Liver / Brain	20	590.555	20	653.141	20	701.561*	20	770.798*	20	809.190*	---->
+Kidneys	20	14.445	20	2.895	20	3.050	20	3.077	20	3.162*	---->
Kidneys / Body	20	3.064	20	0.625	19	0.651	20	0.644	20	0.656	
Kidneys / Brain	20	590.555	20	117.954	20	124.724	20	127.914*	20	129.138*	---->
+Adrenals	20	0.070	20	0.070	20	0.069	20	0.072*	20	0.070	
Adrenals / Body	20	0.0152	20	0.0154	20	0.0145	20	0.0149	20	0.0153	
Adrenals / Brain	20	2.880	20	2.905	20	2.831	20	3.020*	20	3.010	
+Thymus	20	0.413	20	0.395	20	0.418	20	0.506*	20	0.446	
Thymus / Body	20	0.090	20	0.086	19	0.087	20	0.105*	20	0.093	
Thymus / Brain	20	16.984	20	16.215	20	17.181	20	21.037*	20	18.304	
+Gonads	20	3.649	20	4.005	20	4.041*	20	4.048*	20	4.146*	
Gonads / Body	20	0.784	20	0.868	19	0.853	20	0.850	20	0.863	
Gonads / Brain	20	149.165	20	163.055	20	165.401*	20	168.018*	20	169.600*	
+Spleen	20	0.715	20	0.727	20	0.717	20	0.715	20	0.658	
Spleen / Body	20	0.155	20	0.156	20	0.153	20	0.150	20	0.137	<----
Spleen / Brain	20	29.391	20	29.592	20	29.260	20	29.738	20	26.951	-
+Thyroid	20	0.0444	20	0.0391*	20	0.0369*	20	0.0509	20	0.0493	---->
Thyroid / Body	20	0.0096	20	0.0084	20	0.0078*	20	0.0106*	20	0.0102	
Thyroid / Brain	20	1.819	20	1.593	20	1.512	20	2.119	20	2.020	---->

NO. = NO. OF VALUES/GROUP

* = SIGN. DIFFERENCE (LOCATION AND/OR DISPERSION) BETWEEN CONTROL (GROUP 1) AND GROUP X (SIGN. L. = 0.050)

----> = SIGN. POS. TREND FROM CONTROL TO HIGHEST DOSAGE –GROUP (SIGN. L. = 0.010)

<---- = SIGN. NEG. TREND FROM CONTROL TO HIGHEST DOSAGE –GROUP (SIGN. L. = 0.010)

Mean Organ Weight and Ratios (As a Percentage of Body - and Brain Weight)

Table 2
Data for Female Rats

ORGANS	DOSE IN PPM										
	0.0		60.0		200.0		600.0		2000.0		TREND
	NO.	MEAN	NO.	MEAN	NO.	MEAN	NO.	MEAN	NO.	MEAN	
+Body	20	288.155	20	290.864	20	287.904	20	290.679	18	281.688	
+Brain	20	2.267	20	2.263	20	2.303	20	2.283	19	2.252	
Brain / Body	20	0.792	20	0.785	19	0.804	20	0.792	18	0.805	
+Heart	20	1.001	20	0.957	20	0.963	20	0.942	19	0.935	
Heart / Body	20	0.348	20	0.331	20	0.336	20	0.325*	18	0.332	
Heart / Brain	20	44.228	20	42.327	20	41.861	20	41.280*	19	41.645	<----
+Liver	20	9.745	20	10.112	20	10.210	20	10.502	19	11.213*	---->
Liver / Body	20	3.377	20	3.492	20	3.554	20	3.615*	18	4.031*	---->
Liver / Brain	20	430.191	20	447.227	20	443.467	20	460.132	19	498.544*	---->
+Kidneys	20	2.021	20	1.920	20	2.069	20	1.934	19	1.940	
Kidneys / Body	20	0.700	20	0.665	20	0.720	20	0.669	18	0.697	
Kidneys / Brain	20	89.172	20	84.870	20	89.851	20	84.775	19	86.365	
+Adrenals	20	0.092	20	0.086	20	0.086	20	0.092	19	0.090	
Adrenals / Body	20	0.0318	20	0.0295	20	0.0301	20	0.0320	18	0.0317	
Adrenals / Brain	20	4.065	20	3.783	20	3.734	20	4.040	19	4.002	
+Thymus	20	0.372	20	0.334	20	0.330	20	0.379	19	0.351	
Thymus / Body	20	0.128	20	0.114	20	0.115	20	0.131	18	0.125	
Thymus / Brain	20	16.409	20	14.782	20	14.350	20	16.595	19	15.618	
+Gonads	20	0.183	20	0.169	20	0.162	20	0.201	19	0.182	
Gonads / Body	20	0.064	20	0.059	20	0.057	20	0.070	18	0.065	
Gonads / Brain	20	8.099	20	7.520	20	7.084	20	8.836	19	8.124	
+Spleen	20	0.502	20	0.488	20	0.490	20	0.518	19	0.525	
Spleen / Body	20	0.174	20	0.168	20	0.171	20	0.179	18	0.189	
Spleen / Brain	20	22.191	20	21.587	20	21.297	20	22.675	19	23.371	
+Thyroid	19	0.0359	20	0.0291*	20	0.0328	20	0.0366	19	0.0386	---->
Thyroid / Body	19	0.0126	20	0.0100*	20	0.0114	20	0.0127	18	0.0139	---->
Thyroid / Brain	19	1.584	20	1.286*	20	1.425	20	1.610	19	1.716	---->

NO. = NO. OF VALUES/GROUP

* = SIGN. DIFFERENCE (LOCATION AND/OR DISPERSION) BETWEEN CONTROL (GROUP 1) AND GROUP X (SIGN. L. = 0.050)

----> = SIGN. POS. TREND FROM CONTROL TO HIGHEST DOSAGE –GROUP (SIGN. L. = 0.010)

<---- = SIGN. NEG. TREND FROM CONTROL TO HIGHEST DOSAGE –GROUP (SIGN. L. = 0.010)

	Histopathology: Apart from minimal hypertrophy in the centrilobular region of the liver (20/20 males and 3/19 females at 2000 ppm, and 6/20 males at 600 ppm), no macroscopic or microscopic findings were present that could be considered due to the administration of test substance. The NOEL is 60 ppm, corresponding to a mean daily intake of 4.4 mg/kg bw of test substance for males and 4.5 mg/kg bw for females..
Remarks:	This study was assigned a reliability code of 1a[2] (guideline study).
Reference:	[1] 3 Month Toxicity Study in Rats, Final Report, July 4, 1984. GU project no. 820112, Ciba Geigy Limited, Basel, Switzerland. [2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.